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In vitro study of antibacterial activity of the alga *Sargassum oligocystum* from the Persian Gulf

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Abstract. – *Background and Objectives:* With due attention to the development of drug-resistant bacteria, discovering of new antibacterial compounds is needed. Algae produce numerous bioactive substances which may have pharmacological properties such as antibacterial activity. The objective of this investigation was to *in vitro* study of antibacterial activity of brown alga *Sargassum oligocystum* collected along the Bushehr coast of Persian Gulf (south west of Iran).

***Materials and Methods:* Hot water extract, cold water extract, and hot glycerin extract were prepared. The effect of the extracts were investigated on *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922).**

***Results:* Hot water extract exhibited antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. Cold water extract and hot glycerin extract did not show antibacterial activity on any of the four test bacteria. The minimum inhibitory concentration (MIC) of hot water extract for both *Staphylococcus aureus* and *Staphylococcus epidermidis* was 3.175 mg/ml. However, the MIC of this extract for *Pseudomonas aeruginosa* was 9.556 mg/ml.**

***Discussion:* In this study gram-positive bacteria were more susceptible to hot water extract than gram-negative bacteria. Extract of *Sargassum oligocystum* could be a candidate for purification and further *in vivo* studies.**

Key Words:

Antibacterial activity, *Sargassum oligocystum*, Water extract, Glycerin extract, Alga.

Introduction

Bacterial infections are among worldwide and important diseases that cause high rate mortality in humans. Antimicrobial agents are commonly used in treatment of bacterial infections. However, bacteria can become resistant to available drugs[1]. Therefore, discovering of new antibacterial compounds is required. Nowadays, increasing popularity of traditional medicine has led researchers to investigate the natural compounds in plants and algae[2,3].

Marine algae are among the major producer of biomass and have vast ecological importance in their environment. Some algae have been used as human food source in the Asia diet, since these organisms contain essential nutrients[4]. For example, it has been shown that *Kappaphycus alvarezzi*, an edible seaweed from the west coast of India, contains a significant amount of fibers, proteins, carbohydrates, unsaturated fatty acids, and minerals[5]. Also algae have been recognized as the rich source of bioactive natural products which may have properties such as antioxidant[5,6], antiulcer[7], anticancer[8], antifungal[9,10], antiprotozoal[11], antiviral[12,13], or antibacterial activity[3,4,9,14,15].

Sargassum oligocystum is a brown alga that falls into the Sargassaceae family; the Persian Gulf is a rich source for this alga. In previous article, we reported the anticancer effect of *Sargassum oligocystum* against two human cancer cell lines[16]. The present investigation was aimed to *in vitro* study of antibacterial activity of *Sargassum oligocystum* from the Persian Gulf.

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Materials and Methods

Preparation of Algal Extracts

Sargassum oligocystum was collected from the Bushehr coast of Persian Gulf (south west of Iran) in October 2007. Algal sample was thoroughly washed with distilled water to remove epiphytes and any associated debris. In this study, three kinds of crude extracts were prepared that were hot water extract[17], cold water extract[12], and hot glycerin extract[18]. To prepare hot water extract, 20 g of fresh alga was homogenized in 200 ml double distilled water and boiled for 20 minutes. Thereafter, the mixture was clarified using filtration with Wathman paper No. 1. Preparation of cold water extract was performed similar to that described above, but boiling step was omitted. Hot glycerin extract was prepared similar to hot water extract preparation except that algal sample was homogenized in 20% glycerin solution instead of double distilled water. Sterilization of the extracts was carried out by using filter with 0.22 μm pore size.

Bacterial Strains

The American Type Culture Collection (ATCC) strains in this investigation were *Staphylococcus (S) aureus* (ATCC 25923), *Staphylococcus (S) epidermidis* (ATCC 14990), *Pseudomonas (P)aeruginosa* (ATCC 27853), and *Escherichia (E) coli* (ATCC 25922) which were used as test microorganisms. To store the bacterial strains, maintenance procedure was done as follows: the bacteria were cultured on brain heart agar (Merck, Darmstadt, Germany) and incubated at 37ºC. Thereafter, the grown colonies were harvested from the culture medium, suspended in skim milk (Merck, Darmstadt,Germany) containing 10% glycerol (Merck, Darmstadt,Germany) and kept[19] at –20ºC.

Antibacterial Activity Test

Fresh pure cultures of bacterial strains, outlined above, were prepared and antibacterial effect of hot water extract against each strain was tested as follow: in the test tube containing Mueller Hinton broth (Merck, Darmstadt, Germany), a microbial concentration of 5×10^5 colony forming units (CFU)/ml[20] was examined with 6.35 mg/ml of the extract. In addition, a tube of the Mueller Hinton broth containing the same bacterial concentration, but without the extract, was utilized as growth control. Another tube of the uninoculated Mueller Hinton broth containing the same concentration of the extract was used as negative growth control[20]. The test tube and two control tubes were incubated at 37ºC for 24 hours. After incubation, antibacterial activity of the extract in the test tube was detected by lack of turbidity (matching the negative growth control) which indicating the inhibition of bacterial growth[20,21].

Examination of cold water extract was also performed by the same way. The concentration of this extract in the test tube was 3.8 mg/ml because the concentration of original cold water extract was lower than the concentration of original hot water extract.

In the next step of the work, both hot water and cold water extracts were examined at a higher concentration in the test tubes so that the concentrations of hot water extract and cold water extract were 11.316 mg/ml and 6.772 mg/ml, respectively. This step was performed for those test organisms which had not been inhibited with 6.35 mg/ml of hot water extract or 3.8 mg/ml of cold water extract.

Examination of hot glycerin extract was carried out similar to described above. In the test tube, 5×10^5 CFU/ml of each organism was examined with 4.76 mg/ml of hot glycerin extract. Besides growth control and negative growth control, an additional tube of Mueller Hinton broth containing the same bacterial concentration and sterile glycerin solution, but without extract, was prepared to control and rule out antibacterial effect of glycerin solution. Incubation of tubes and detection of results were done as described above.

Determination of Minimum Inhibitory Concentration (MIC)

Since hot water extract showed antibacterial activity on *S. aureus*, *S. epidermidis*, and *P. aeruginosa*, the MIC of this extract for mentioned bacteria was determined using broth dilution method[20,21]. To determine MIC for *S. aureus* and *S. epidermidis*, the extract was added as serial dilutions to a series of tubes containing Mueller Hinton broth so that the concentrations ranged from 6.35 to 0.024 mg/ml. The bacterial concentration in each tube was 5×10^5 CFU/ml. Growth control and negative growth control were also prepared. For determination of MIC for *P. aeruginosa*, the extract was added to several tubes containing Mueller Hinton Broth so that the concentrations ranged from 11.316 to 6.287

mg/ml. After 24 hours incubation at 37ºC, the lowest concentration of extract that led to inhibit the growth of bacteria, was considered as MIC.

Statistical Analysis

The experiments were replicated three times. Statistical analysis was performed using SPSS software version: 16. For data analysis, Mann-Whitney U test was used.

Results

The results of antibacterial activity assay of hot water, cold water and hot glycerin extracts on *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 14990), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922) are summarized in Table I. In the step of antibacterial activity test, 6.35 mg/ml of the hot water extract was effective against *S. aureus* and *S. epidermidis*, whereas it did not exhibit antibacterial activity on *P. aeruginosa* and *E. coli*. By increasing the concentration of hot water extract in the test tube, we found that 11.316 mg/ml of this extract is effective against *P. aeruginosa*, while it did not show antibacterial activity on *E. coli*. Both 3.8 and 6.772 mg/ml of the cold water extract as well as 4.76 mg/ml of the hot glycerin extract were not found to be effective against the four bacterial strains.

The positive results in the antibacterial activity test were then examined to determine MIC (Table I). The MIC of hot water extract for both *S. aureus* and *S. epidermidis* was 3.175 mg/ml. However, the MIC of this extract for *P. aeruginosa* was higher (9.556 mg/ml). A significant difference was found between the MIC of *P. aeruginosa* and MIC of *S. aureus* or S. *epidermidi*.

Discussion

The algae belong to a group of organisms that produce numerous secondary or primary metabolites[22,23]. Bioactive compounds derived from algae are brominated, aromatics, nitrogen-heterocyclic, sterols, proteins, peptides, and sulfated polysaccharides[24]. These bioactive substances may have pharmacological properties such as antibacterial activity[22-24]. Thus, there is an increasing interest to evaluate the algae for their antibacterial effects. An important role of such compounds can be the protection of algae against other organisms[9,22]. We had access to *Sargassum oligocystum* on the Bushehr coast and designed this study to investigate its antibacterial activity.

In this study, the crude extracts of *Sargassum oligocystum* were examined on *S. aureus* and *S. epidermidis* as two gram-positive as well as *P. aeruginosa* and *E. coli* as two gram-negative bacteria. These bacterial species are representative of the important pathogens in humans and were used in many investigations related to finding antibacterial compounds[4,10,22,25].

We used fresh alga sample for preparation of algal extracts. Tüney et al[15] have shown that extracts prepared from fresh algal samples possess

Table I. Examination of hot water, cold water, and hot glycerin extracts for evaluation of antibacterial activity and determination of MIC.

Bacteria	Extracts	Antibacterial activity	MIC (mg/ml)
S. aureus ATCC 25923	Hot water extract	+	3.175 ± 0.064
	Cold water extract	–	
	Hot glycerin extract	–	
S. epidermidis ATCC 14990	Hot water extract	+	3.175 ± 0.000
	Cold water extract	–	
	Hot glycerin extract	–	
P. aeruginosa ATCC 27853	Hot water extract	+	9.556 ± 0.251
	Cold water extract	–	
	Hot glycerin extract	–	
E. coli ATCC 25922	Hot water extract	–	
	Cold water extract	–	
	Hot glycerin extract	–	

+: Shows activity; –: No activity; Each MIC represents mean ± standard deviation (SD).

more antibacterial activity than extracts from air-dried algae. The reason might be the loss of some bioactive compounds in the drying process.

In different investigations a variety of solvents have been used for extraction of antimicrobial substances from algae. However, it is still uncertain what kind of solvent is the most effective for this extraction[10]. Hot water and cold water extracts were prepared in our study, since in other investigations both kinds of extracts had shown an acceptable antiviral activity[12,17]. Another reason for choosing of these extracts was the fact that the majority of traditional medicines are prepared using water[26]. Hot water extract exhibited antibacterial activity against *S. aureus*, *S. epidermidis*, and *P. aeruginosa*, whereas cold water extract did not show antibacterial effect on any of the four test organisms. Therefore, it could be conclude that, firstly, *Sargassum oligocystum* contains antibacterial compounds which can be extracted by hot water, but not by cold water, and secondly, that these compounds are heat stable.

Hot glycerin extract was the third extract which we used in the present study because in a previous investigation, hot glycerin extract of *Avicennia marina* had shown antibacterial effect against *S. aureus*, *P. aeruginosa*, and *E. coli*[27]. Also, in another study[18], it has been shown that hot glycerin extract of *Aloe vera* had antiviral activity against herpes simplex virus type 2. However, in the present study hot glycerin extract was not found to be effective against test bacteria. So, it seems that glycerin solution is not suitable for extraction of antibacterial substances from *Sargassum oligocystum*. This might be due to diversity of bioactive constituents in different organisms.

As shown in Table I, hot water extract had antibacterial effect on both *S.aureus* and *S. epidermidis* (gram-positive species), whereas out of two gram-negative species, only *P. aeruginosa* was inhibited by this extract. In addition, the MIC for *S. aureus* and *S. epidermidis* was lower than the MIC for *P. aeruginosa*. Therefore, in this study, gram-positive species were more susceptible to hot water extract than gram-negative species. Discrepancy in the antibacterial activity of the extract against gram-positive and gram-negative species may be due to structural differences which influence the cell envelope permeability[28].

Sandsdalen et al[22] found an antibacterial compound in the brown alga, *Fucus vesiculosis* which showed activity against several test organisms such as *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*. Further examinations indicated that the antibacterial activity was caused by a polyhydroxylated fucophlorethol. In the investigation conducted by Ibtissam et al[29], crude methanol extract of *Sargassum vulgare* did not show antibacterial activity on *S. aureus*, *E. coli* and other test bacteria, whereas in our study hot water extract exhibited activity against three test bacteria. Antibacterial properties of crude methalonic extract of *Sargassum myricocystum* and *sargassum tenneerimum* was evaluated by Kandhasamy el al[25]. Similar to our results, extract of *Sargassum myriocystum* was effective against *S. aureus* and *P. aeruginosa*, but it did not exhibit activity against *E. coli*. Whereas, contrary to our results, extract of *Sargassum tenneeriumum* was also effective against *E. coli*. These Authors also concluded that gram-positive bacteria were more susceptible than gram-negative bacteria in their study. Ravi Kumar et al[28] did not find antibacterial properties in the crude methalonic extract of *Sargassum wightii*. It is understood from the articles, outlined above, that there are both similarities and differences between their results and our results. These differences may be due to some factors such as possible dissimilarities in various species or genera of algae for production of metabolites, seasonal variations, and differences in solvents used for extraction[15,25].

When the present work was performed and this article was drafted, then we found a Meeting Abstract concerning investigation of antibacterial effect of *Sargassum oligocystum* from Philippines against some aquaculture pathogenic bacteria such as *Vibrio* species[30]. The methanol extract showed antibacterial activity. But, in contrast to our study, antibacterial activity has not been reported for aqueous extract. Since mentioned report was only as an abstract which normally lack of details in methods and results, we were not able to present a complete comparison of its results with our results. But, it is obvious that there are differences in geographical region and habitat as well as test bacteria between mentioned work and our study which could influence the results. It has been confirmed that the antibacterial properties in the same species may vary by habitats, life phase, and seasonal variations[22]. Moreover, extraction procedure is an important factor[15].

There are several studies concerning investigation of antibacterial activity of the same species of algae in different parts of the world or even in different regions of a country. For example, alga

Ulva fasciata from Brazilian coast[23] and from the coast of Morocco[29] or alga *Ulva rigida* from the coast of Urla, Izmir, Turkey[15] and from the Aegean sea, Turkey[3] were investigated.

In conclusion, *Sargassum oligocystum* could be a good source for antibacterial compounds. It could be a suitable candidate for purification of crude extracts and further *in vivo* studies.

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